ABSTRACT OF THE DISCLOSURE

Methods and compositions for producing single-stranded cDNA (ss-cDNA) with a vector-based system in eukaryotic cells. In one embodiment, the vector comprises plasmid(s) that contain a reverse transcriptase/RNAse H gene and a cassette, which includes a sequence of interest and an inverted repeat, which produces an RNA template from which the reverse transcriptase synthesizes ss-cDNA of a specified sequence. The ss-cDNA is then modified to remove all flanking vector sequences by taking advantage of the "stem-loop" structure of the ss-cDNA, which forms as a result of the inclusion of an inverted tandem repeat that allows the ss-cDNA to fold back on itself, forming a double stranded DNA stem with the sequence of interest in the loop portion of this intermediate. The double-stranded stem may also contain one or more restriction endonuclease recognition sites and the double-stranded stem of the stem-loop intermediate is cleaved by the desired corresponding restriction endonuclease(s) so that the loop portion, or sequence of interest, is then released as a linearized, single-stranded piece of DNA. The plasmid may also include a gene for producing the restriction endonuclease specific for this site in the stem. This released ss-DNA sequence contains minimal sequence information either upstream 5' or downstream 3' from the previous double stranded stem portion which contains the restriction endonuclease cut site. The plasmid also includes a second sequence of interest 3′ to the inverted repeats which is likewise produced with minimal vector sequence.

In vivo transfections using the DNA vector constructs described herein demonstrate the use of this system to produce ss-DNA in eukaryotic cells by taking advantage of the many potential promoter(s)/enhancer(s) signals, polyadenylation signals, splice site junctions, ribosome binding sites, and origin of replication signals known to those skilled in the art. The experiments described herein show expression of reverse transcriptase(s)/RNase H(s) within eukaryotic cells as well as synthesis of RNA transcripts which serve as the template directing the formation of the ss-cDNA for such therapeutic purposes as gene inactivation using duplex or triplex binding of nucleic acids, site-directed mutagenesis, interruption of cellular function by binding to specific cellular proteins, and interfering with RNA splicing functions.